T H E S I S

for the Degree of M.D. (Edin.)

On ISOAGGLUTINATION in HUMAN BLOOD

with some General Observations

on AGGLUTINATION PHENOMENA

by

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In the Journal of Mental Science, July 1906, Dr Lewis C. Bruce drew attention to an agglutinating reaction between the serum of patients suffering from certain infective diseases and normal washed red blood corpuscles. His technique, as he described it to me in 1907 is as follows:-

The serum of the patient to be examined is obtained in the usual way. A few drops of blood are allowed to flow into a glass pipette from a puncture on dorsum of a finger just above the nail root, - the pipette is sealed at both ends and the serum separated from the clot by centrifuging for a few minutes.

The washed red blood corpuscles are obtained from a healthy individual, whose serum has been previously tested for this reaction and found negative. A few drops of his blood obtained from finger are mixed with three or four times their volume of a one per cent solution of sodium citrate in normal saline. After a thorough shaking the mixture is centrifuged and the clear supernatant fluid pipetted off. The residue is then twice washed with normal saline, centrifuged and supernatant clear fluid pipetted off. Finally the residue is diluted with two or three times its bulk of normal saline.

The Reaction - one drop of the patient's serum is placed in the well of a hanging drop slide and beside it two drops of the washed red blood corpuscles

of healthy individual. The three drops are then mixed together with a platinum loop previously sterilized in the flame of a spirit lamp and cooled. In cases which give the reaction the red blood corpuscles run together in a very few minutes, forming clumps often quite visible to the naked eye and readily confirmed by examination under the low power of a microscope.

Dr Bruce suggested that I should make a series of observations on this reaction amongst my patients in general practice and compare and contrast them with the results obtained by him in asylum practice. To this end he kindly placed at my disposal the clinical records of the Perth District Asylum bearing on the subject and also devoted some of his valuable time to introducing me in his Laboratory to the necessary technique.

I there learnt also the method of estimating the leucocytosis as well as the differential count of the various kinds of leucocytes, both being important points in the investigation of the blood in infective disorders.

Having tested my own blood serum with the washed erythrocytes of several healthy individuals, I always got a negative reaction. I adopted it as a standard, and in the course of all the subsequent investigations used my own blood from which to prepare the washed erythrocytes for mixing with the serum of the cases

tested. I repeated several of Dr Bruce's experiments and observations regarding the general nature of the reaction. Some of these were carried out in the Asylum Laboratory and others in spare time at home. These preliminary observations relate to such considerations as -

(1) The effect of heat on the activity of the agglutinating agent in a serum.

(2) The effect of time, determining whether an agglutinating serum lost any of its agglutinating power by keeping.

(3) Whether the agglutinating power of a serum could be exhausted by the agglutinating of the washed erythrocytes or could be used over again to agglutinate a fresh supply of erythrocytes.

(4) The effect of crossed reactions between two patients, both of whose sera gave the agglutinating reaction, mixing the serum of one such patient with the washed erythrocytes of another such and vice versa.

Taking these seriatim:-

(1) <u>The effect of heat</u>.

I took a small quantity of blood in two glass pipettes from Maggie P. (Case No.1 in the following series) a rheumatic patient.

After centrifuging both, I mixed one drop of the serum from one of the pipettes with two drops of normal washed erythrocytes. The result was an immediate

and well marked agglutination.

The other pipette of centrifuged blood I placed in my dry air sterilizer, raised the temperature to 60° Centigrade, and kept it there for 30 minutes. Thereafter on testing a drop of the serum in the same way as before, the agglutinating reaction was as rapid and marked as before, showing that the agglutinating power of the serum had not been affected by the heat; that is to say that the agglutinating agent is thermostable.[1]

(2) The effect of time.

On 24th September 1907 I took some blood in 4 pipettes from Maggie P. (Case 1 in series) a rheumatic patient, and centrifuged them. On four subsequent dates, 26th and 29th September and 1st and 3rd October I took one of the pipettes in series and tested the serum with freshly prepared corpuscles and on each occasion got a well marked agglutination. The serum examined on 3rd October had thus been kept nine days and apparently had lost none of its agglutinating power. I subsequently repeated this experiment on more than one occasion, keeping the serum as long as three weeks and still finding at the end of that time that the agglutinating agent was quite active.

Footnote 1.
Dilution experiments would have modified this conclusion somewhat. Fleming[5] in an article subsequently referred to, pointed out that though heating did not alter the character of the haemoagglutinin in a given serum it did alter its strength. For example a serum which before heating produced agglutination in a certain sample of washed corpuscles even when diluted 64 times, after heating failed to produce agglutination if diluted 4 times, though the reaction was still well marked with the undiluted serum.

(3) Experiments on the exhaustion of the agglutinating power of a serum giving the reaction.

(a) Serum of M.I., a case of confusional mania mixed with normal washed erythrocytes in the usual proportions gave a marked agglutination.

(b) Washed erythrocytes of M.I. whose serum gave the reaction, when mixed with normal washed red cells gave no reaction, - shewing that the red cells of a case which gives the reaction contain none of the agglutinating agent.

(c) Serum of M.I. was mixed with normal washed reds in equal proportions, well shaken together, and allowed to stand for ten minutes, then centrifuged and supernatant fluid pipetted off.

This fluid mixed with a fresh supply of control reds gave no reaction, shewing that the serum had been exhausted of its agglutinating agent in the first mixing.

(d) One part of the above-mentioned exhausted serum from M.I. was mixed with one part normal serum and one part control erythrocytes. The result was negative, shewing that the exhausted serum acquired no help or rehabilitation of its agglutinating power from mixture with normal serum.

(e) Serum of M.I. was mixed with an equal quantity of washed erythrocytes, shaken together, centrifuged and supernatant fluid pipetted off. The

residue was mixed with three parts distilled water allowed to stand fifteen minutes, centrifuged and supernatant fluid pipetted off. This supernatant fluid was mixed with an equal quantity of double strength saline fluid.

Eight parts of this haemolysed red solution to one part control reds gave a slight reaction, shewing some recovery of agglutinating power from the haemolysing of agglutinated reds.

(f) Eight parts of above described haemolysed red solution and one part control reds were mixed, with the addition of one part normal serum but with no increase of the slight reaction previously obtained, confirming the conclusion of experiment (d) that normal serum adds nothing to the agglutinating power of a serum whose haemoagglutinin has been previously exhausted or reduced by the act of agglutinating red cells.

(4) Experiments in crossed reactions.

(a) Control serum of sane person, which gave a marked reaction with normal reds, was mixed with washed red cells from a case of mania, whose serum also gave the reaction with normal reds. The result was negative.

(b) Above mixture of two parts serum and one part reds was allowed to stand for five minutes, and there still being no reaction, one part normal

washed reds was added to it. The result was an immediate agglutination of a great portion of the reds, the action being apparently selective, the agglutinin picking out the normal reds and leaving the others unaffected.

(c) This experiment was repeated with serum from one case of mania and washed reds from another case of the same disease, the serum of both cases having previously given a marked agglutination with normal reds. The result was entirely negative; but on the addition of one part normal reds there was an immediate clumping, but again, as in experiment (b) the action was selective, the normal reds probably being alone affected, those from the case of mania going free.

Various control and cross reactions investigated during this research and jotted down in my notebook.

(a) On 17th September 1907, serum and washed erythrocytes were prepared from three individuals, viz:- Mrs L., a patient convalescent from Pneumonia and Phlebitis, and two healthy individuals, A. and J.

Mrs L's serum to J.'s corpuscles - reaction positive.
Mrs L's serum to A.'s corpuscles - reaction positive.
Mrs L's serum to Mrs L's corpuscles - reaction negative.
J.'s serum to Mrs L's corpuscles - reaction negative.
J.'s serum to A.'s corpuscles - reaction negative.
J.'s serum to J.'s corpuscles - reaction negative.

A.'s serum to Mrs L's corpuscles - reaction negative.

A.'s serum to J.'s corpuscles - reaction negative.

A.'s serum to A.'s corpuscles - reaction negative.

(b) On 19th September 1907 serum and washed corpuscles were prepared from (1) D.R., an apparently healthy person whose serum nevertheless always gave an agglutinating reaction, from (2) R.L. a boy convalescent from an attack of gastric intestinal catarrh with fever, from (3) Mrs L. convalescent from pneumonia and phlebitis, and (4) washed corpuscles from control A whose serum never gave agglutinating reaction though frequently tested.

Mrs L.'s serum to A.'s corpuscles - reaction positive.

D.R.'s serum to A.'s corpuscles - reaction positive.

R.L.'s serum to A.'s corpuscles - reaction positive.

Mrs L.'s serum to D.R.'s corpuscles - reaction negative.

D.R.'s serum to Mrs L.'s corpuscles - reaction negative.

R.L.'s serum to D.R.'s corpuscles - reaction negative.

R.L's serum to Mrs L.'s corpuscles - <u>reaction positive</u>!

(c) On 23rd September 1907, serum was prepared from cases Mrs L. above mentioned and from K.D., a case of pernicious (?) anaemia, washed corpuscles from R.L. also mentioned above, and J. furnished the control reds.

Mrs L.'s serum to J.'s corpuscles - reaction positive.

Mrs L.'s serum to R.L!s corpuscles - reaction negative.

K.D.'s serum to J.'s corpuscles - reaction negative.

K.D.'s serum to R.L.'s corpuscles - reaction negative.

R.L.'s serum to J.'s corpuscles - reaction negative.

(d) On 11th October 1907, serum and corpuscles were again prepared from Mrs L., and from A.H. a healthy young woman but the subject of an extensive infective history in the past, including dysentery, enteric fever and blood poisoning from an infected wound.
J. again furnished the control reds.
Mrs L.'s serum to J.'s corpuscles - reaction positive.
A.H.'s serum to J.'s corpuscles - reaction positive.
A.H.'s serum to Mrs L.'s corpuscles - reaction negative.
Mrs L.'s serum to A.H!s corpuscles - <u>reaction positive</u>!

(e) On 20th October, 1907 serum and corpuscles were prepared from H.R. a convalescent from appendicitis, and from N.M. an apparently healthy youth with a history of gastric fever some ten years ago. J. again furnished the control reds.
H.R.'s serum to J.'s corpuscles - reaction positive.
N.M.'s serum to J.'s corpuscles - reaction positive.
H.R.'s serum to N.M.'s corpuscles - reaction negative.
N.M.'s serum to H.R.'s corpuscles - reaction negative.

Thus on two occasions (underlined), even in the above limited number of cases, I obtained agglutination between two bloods both of whose sera had given the reaction with normal washed reds (interagglutination.)

This interagglutination, Dr Bruce stated (op.cit.) would never occur. If, however, the factors in the reaction (the agglutinating power of the serum and the

agglutinability of the corpuscles) are variable in quantity or quality or both, one would expect such cross reactions to be fairly frequent. This variability in the factors I found on subsequent knowledge to be an undoubted fact. In considering later the researches of previous workers in this subject of isoagglutination (chiefly German and dating from 1900) I shall indicate that over 90% of normal bloods contain haemoagglutinin, and further that in about 50% of normal bloods the phenomenon of interagglutination can also be shown.

This, of course, enormously diminishes the possibility of any diagnostic value attaching to the phenomenon; if indeed it does not dispel it altogether.

I now append ten cases taken from my notebook of patients who were under more or less continuous observation for varying lengths of time and in whom I made a series of detailed blood examinations and also the above described agglutination test.

In each I give a short and very general history of the case, sufficient only to indicate its nature.

They are all cases which are pretty well recognized nowadays as infective, that is to say, caused by microbic invasion or by the absorption of toxic products of microbic activity in the tissues or cavities of the body.

1. Maggie P., aet 19, a case of rheumatic chorea, first attended 4th April, 1907, complaining of pains in joints and twitching of arms and legs - fever moderate 100° - 101° Fah. No heart complications - treated with solicylates and arsenic. Improvement very gradual. On 24th April gave a hypodermic injection of pure Terebene (m 3) with a view to raise the abnormally small leucocytosis. On 31st August saw patient and noted that she had still some slight chorea in eye muscles and occasional twinges of pain in both shoulders and in right wrist. No heart murmurs - menstruation irregular. Agglutinating reaction strongly marked in every observation made, seven in number, from 26th April to 24th Septr.

Date of Observation	4 April 07	15 April 07	26 April 07	30 April 07	4 May 07	12 May 07	22 May 07	31 Aug.07	24 Sep.07
Agglutinating Reaction.			+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Leucocytes	8,200	4,000	16,200	27,000	16,300	18,200	13,000	12,300	9,500
Differential Count									
Polymorphs	46.6%	30%		47%	33%	48%	29%		
Small Lymphocytes.	32	24		15	32.75	28.75	27.8		
Large Lymphocytes.	9.8	27.5		20.5	25.5	16.5	29.6		
Hyalines.	8.8	11		12.5	2.25	1.75	5.2		
Eosinophiles	3.0	6.5		3.0	5.75	4.25	7.4		
Mast Cells	0.0	1.0		2.0	.25	.75	1.0		
Haemoglobin	90%		100%	90%	95%				
Erythrocytes	6,128,000	6,352,000	6,928,000	7,464,000,	7,864,000				
Colour Index.	.7		.8	.6	.65				

2. Mr Kenneth D. aet 28 - a case of profound anaemia with marked pallor and with red cells varying from one to three millions per cubic millimetre - apparently an essential or pernicious anaemia but without haemorrhages or even haemic murmurs - no great wasting or shortness of breath or muscular debility and no megaloblasts found in any of the many blood films examined.

This patient is still alive (25 Feb.09) and following his occupation as a watchmaker. His red cells number about three millions per cub.min., and Haemoglobin 50%. His chief treatment has been arsenic. He takes 15 m. of Fowler's solution thrice daily for 2 or 3 weeks at a time without any inconvenience. The agglutinating reaction was always negative on the occasions tested.

Date of Observation	1 April 07	11 April 07	17 April 07	22 April 07	29 April 07	3 May 07	6 June 07	2 July 07	9 July 07	15 July 07
Agglutinating Reaction					Negative	Negative	Negative			
Haemoglobin	50%	55%	56%	55%	50%	50%		40%	40%	35%
Erythrocytes			2,768,000	2,416,000	3,000,000	2,472000	1,608000	772,000	1,304,000	1,664,000
Colour Index			1.03	1.13	.83	1.04		2.6	1.5	1.2
Leucocytes		3,000	4,800	3,000	7,200	4,400		1,800	1,200	1,300
Differential										
Polymorphs	57%	45%	45%	38%	51.5%	65%	29%			
Small Lymphocytes		17.5	40.0	39.0	36.0	23.5	59.0			
Large Lymphocytes	34.5	30.0	13.5	13.0	11.5	8.5	8.5			
Hyalines	5.5	5.0	.5	9.0	0.0	0.5	0.0			
Eosinophiles	3.0	2.5	1.0	1.0	1.0	2.5	3.5			
Masts	0.0	0.0	0.0	0.0	0.0	0.0	0.0			

Continued.

Mr Kenneth D. (Continued.)

Date of Observation	23 July 07	29 July 07	6 Aug. 07	26 Aug. 07	9 Sep. 07	23 Sep. 07	7 Oct. 07	21 Oct. 07	5 Nov. 07	18 Nov. 07	2 Dec. 07
Agglutinating Reaction	Negative	Negative	Negative	Negative	Negative		Negative			Negative	Negative
Haemoglobin.	35%	35%	35%	40%	35%	35%	35%	42%	48%	50%	50%
Erythrocytes	1,388,000	1,140,000	1,256,000	1,880,000	1,600,000	1,108,000	1,708,000	2,116,000	1,920,000	2,832,000	2,940,000
Colour Index	1.2	1.6	1.4	1.1	1.2	1.6	1.03	1.0	1.2	.89	.86
Leucocytes.	2,200	1,300	1,900	3,800	2,200	2,000	4,000	1,700	2,300	3,100	3,400
Differential Count											
Polymorphs				40%	32%			48%			
Small Lymphocytes				34.0	48.0			30.0			
Large Lymphocytes				21.0	20.0			20.0			
Hyalines				0.0	0.0			0.0			
Eosinophiles				4.0	0.0			2.0			
Masts				1.0	0.0			0.0			

3. Miss W. R. aet 35, has some spinal lesion of gradual onset and slow progress causing weakness and defective control of muscles of right arm and right leg, and she suffers from occasional epileptic attacks. Examination of the blood on several occasions revealed a slight anaemia characterised by a deficiency of the Haemoglobin without any diminution of the number of erythrocytes. The agglutination test always gave a negative result.

Date of Observation 29	April 07	6 May 07	13 May 07	4 Sep.07
Agglutination Reaction	Negative	Negative	Negative	Negative
Haemoglobin		60%	60%	
Erythrocytes		5,256,000	6,712,000	
Colour Index		.57	.45	
Leucocytes	11,600	9,200	9,200	8,800
Polymorphs	62.5%	55%	62.5%	67.5%
Small Lymphocytes	23.0	29.5	20.0	18.75
Large Lymphocytes	10.0	12.5	14.0	10.0
Hyalines	0.0	.25	0.0	0.0
Eosinophiles	1.5	1.5	.5	2.5
Masts	3.0	1.25	3.0	1.25

4. Miss M.S. aet 27, a chronic invalid, asthmatic from time to time, and frequently troubled with renal and vesical pain. She has been frequently examined by gynecologists and other specialists in Edinburgh and elsewhere. The latest diagnosis so obtained was a possibility of renal tubercle, though no tubercle bacilli were found. I have repeatedly examined the centrifuged urinary sediment for tubercle bacilli but found none. Her urine is generally clear, of low specific gravity (1010-1015) and low urea excretion (5 - 7 grs. per ounce) and contains no albumen, pus nor blood at any time.

Her blood examination gives a high leucocytosis, a small percentage of polymorphs (30 - 40%), a large percentage of small lymphocytes (38 - 56%), and a high one of eosinophilis (5 - 6%). The agglutination reaction was always negative.

Date of Observation	30 April 07	8 May 07	13 May 07	22 May 07
Agglutination Reaction	Negative	Negative	Negative	
Haemoglobin	90%			
Erythrocytes	6,156,000			
Colour Index	.74			
Leucocytes	24,000	17,800	12,400	22,000
Polymorphs		32.5%	30%	38.5%
Small Lymphocytes		53.0	56.5	33.5
Large Lymphocytes		7.0	7.0	22.0
Hyalines		2.0	0.0	.25
Eosinophiles		5.5	6.0	5.0
Masts		0.0	0.5	.75

5. Mr F., aged 67, first attended 31st April 1907 for an attack of pleurisy with effusion on right side. The illness was of long duration and of chronic or subacute character.

Eight years previously Mr F. had had a similar attack on the left side with similar prolonged course, necessitating frequent tappings and ultimately a resection of rib. He had never completely regained his previous strength, being always somewhat short of breath and easily tired.

In the present attack his temperature was irregular, never above 100° or 101° - sometimes normal for days at a time. His heart sounds were clear, but systole weak. His urine had a good specific gravity 1020 - 1024 and never any albumen or sugar. He had little cough or expectoration - the latter was examined several times for tubercle bacilli but none found. During the month of June he improved and was able to be outside a little, but in July he became weaker and more breathless. His pleura was tapped on three occasions but only small quantities of fluid were obtained - the fluid was slightly cloudy.

As the patient continued to get worse, a surgeon was called in consultation. He advised and carried out a resection of rib on 8th July. At the operation the pleura was found to be enormously thickened and adherent. Some three or four ounces of cloudy serum was evacuated and a drain inserted. For the first day or two after the operation the patient felt a little better, then failed rapidly, dying on the 12th July.

No post mortem examination was permitted. I made examinations of his blood on several occasions during his illness. His leucocytosis was always high, ranging between 17,400 and 25,600. The agglutinating reaction was always negative.

6. Mr L. aged 63, on 29th May 1907, suffered from great pain in the region of the gall bladder and frequent and severe vomiting. His temperature was 102° Fah., - his urine was of a deep brown, almost black, colour, and his skin was deeply jaundiced.

The attack lasted four days and convalescence was slow and gradual. He had had two previous attacks of a similar nature.

His leucocytosis on 30th May was 17,800 per c.m., on 31st 16,500, and on 2nd June it had fallen to 13,400. The polymorph percentage was high - 75 to 80%.

The serum was tested on two occasions for the agglutinating reaction, but the result was always negative. A curious phenomenon however, presented itself. The serum was deeply jaundiced, of a greenish yellow colour, and on mixing it with the washed red blood corpuscles of control, the bile pigment ran into distinct little clumps, and moreover, some of these clumps were of a reddish brown colour and others of a green colour, suggesting bilirubin and biliverdin.

7. Mr A.W., aged 19, a strong healthy looking young man, on 27th July 1907 consulted me about pains in his joints, chiefly the knee joints. The pains were not very severe, but caused him some anxiety as he had a similar, but much worse attack in November 1906, which had confined him to bed for five weeks. On that occasion he had "fever and sweating" and the doctor said that he "nearly had rheumatic fever."

On examining his heart I found a well marked mitral regurgitant murmur. On the present occasion treatment consisted of rest in bed for a few days and ten grain doses of salicylate of soda thrice daily.

This rapidly effected a cure and he had no recurrence of joint pains during the two months that he continued under observation.

I examined his blood on five occasions and each time the agglutinating reaction was negative. This is so far, the only well marked rheumatic case, either acute or chronic, in which I have failed to get the agglutinating reaction.

Date of Observation	27 July 1907	2 Aug. 1907	9 Aug. 1907	6 Sep. 1907	27 Sep. 1907
Agglutinating Reaction	Negative	Negative	Negative	Negative	Negative
Leucocytosis	11,600	17,100	17,700	16,200	13,100
Polymorphs			56%		52%
Small Lymphocytes			25.5		31.75
Large Lymphocytes			15.0		12.25
Hyalines			0.0		.5
Eosinophiles			2.5		3.0
Masts			1.0		.5

8. Mrs L., aet 64, first attended 9th September 1907, suffering from Pneumonia (left Case). Temperature 103, respirations 40. Stout woman with varicose veins which inflamed during convalescence, first in the right leg and subsequently in the left. Past history - she had always been healthy, had frequently suffered from rheumatic pains, but never had rheumatic fever. Agglutinating reaction was always positive.

Date of Observation	9 Sep.07	11 Sep.07	17 Sep.07	19 Sep.07	23 Sep.07	26 Sep.07	8 Octr.07.
Agglutinating Reaction.	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Leucocytosis	28,400	26,400		33,800			20,400
Differential Polymorphs	73%						73.5%
Small Lymphocytes	17.5						13.0
Large Lymphocytes	8.5						12.5
Hyalines	0.0						0.0
Eosinophiles	1.0						.75
Masts	0.0						.25

9. Mr C.R., aet 31, first attended on 9th September 1907. He complained of praecordial pain, faintness and palpitation, apparently the result of a sudden and prolonged exertion chasing sheep. No heart murmurs to be found on examination, but a fast and feeble pulse sometimes intermitting. He gave a history of having had "heart disease" when ten years old, and an attack of rheumatism when fourteen years old, confining him to bed for about ten days. He is a heavy smoker. With rest, complete stoppage of smoking, and some digitalis, he made a good recovery, but it was ten days or a fortnight before he was fit for any work.

I made three observations on his blood on September 9th, 11th and 15th, and on each occasion obtained a marked agglutinating reaction and a leucocytosis considerably above normal.

Date of Observation	9 Sep.07	11 Sep.07	15 Sep.07
Agglutinating Reaction	+ + +	+ + +	+ + +
Leucocytosis	12,800	14,400	13,200

10. Robert L. aet 12, first attended 17th September 1907. He was suffering from headache and sickness and loss of appetite. Temperature 102.6, - tongue furred - urticarial rash on abdomen and thighs. He had been ill for three or four days and his mother attributed his illness to eating unripe fruit. With rest in bed, milk diet and a few powders containing salicylate of soda and rhubarb he was all right in a few days. On September 21st his temperature was normal and his tongue clean.

I examined his blood on two occasions and both times obtained a marked agglutinating reaction - and also some interesting cross reactions (vide supra) with the blood of another patient, a Mrs L., (no relation of his) who was suffering from pneumonia and phlebitis.

Date of Observation	19 Sep. 07	21 Sep. 07
Agglutinating Reaction	+ + +	+ + +
Leucocytosis		12,600.

These cases are:-

I. Rheumatic Chorea - Reaction positive.

II. Profound (Pernicious?) Anaemia - Reaction negative.

III. Chronic Spinal lesion - Reaction negative.

IV. Chronic renal (tubercular?) case - Reaction negative.

V. Chronic Pleurisy with effusion - Reaction negative.

VI. Cholecystitis with Jaundice - Reaction negative.

VII. Rheumatism subacute - Reaction negative.

VIII. Acute Pneumonia and Phlebitis - Reaction positive.

IX. Rheumatic Heart - Reaction positive.

X. Gastro-intestinal Catarrh - Reaction positive.

Of the ten cases only four give a positive reaction and yet the other six are certainly infective cases in the above mentioned definition of the term; and many of them had a constantly high leucocytosis which is generally regarded as an index of the severity of an infection or intoxication.

One point was, I think, worthy of note, namely in any individual patient the reaction was always the same - if once positive always positive, if once negative always negative.

If found positive, e.g. during an acute illness, it remained so after the patient had recovered and even months afterwards.

This, of course, might be due to a permanent or lasting effect produced by the disease on the patient's

serum. It is well known that typhoid fever patients, e.g. may give a well marked Gruber-Widal reaction long after recovery from the disease.

On the other hand this haemoagglutinin reaction might have been present before the illness, and be quite unaffected by the illness, and indeed have nothing to do with it in a causal relation.

This lead me to make a series of observations on healthy people. This I carried out in family groups so as to throw some light at the same time on another question which had occurred to me - namely - whether or not the phenomenon might be a congenital or hereditary characteristic.

Annexed will be found the details of six such family groups with notes on each individual, of age, infective history, present health, and result of agglutination test as between their serum and my own washed red cells.

1. Family Group - McG's.

	Age	Infective History	Present Health.	Agglutinating Reaction.
Mr McG.	61	Had chicken-pox, measles and hooping cough in infancy, and gastric fever at age 20 but neither Scarlatina nor Rheumatism.	Good	Positive.
Mrs McG.	52	Had measles and chicken pox in childhood and gastric fever at age 10, but neither Scarlatina nor Rheumatism.	Good	Positive.
Mr N.McG.	18	Had gastric fever when aged 4 and Scarlatina and measles subsequently.	Good	Positive.

2. B. Family.

	Age	Infective History.	Present Health	Agglutinating Reaction
Mr B.	79	Had measles, hooping cough and Scarlatina in childhood and a severe bilious attack (?) when 23 years old, lasting six weeks. From age 4 to 12 had running sore from diseased bone in right thigh, and 19 years ago had a threatening of Rheumatic fever.	Good for a man of his age.	Positive but feeble.
Mrs B.	57	Had measles in childhood otherwise been always healthy.	Good	Negative.
Wm.B.	15	Had measles, Scarlatina and hooping cough in childhood and mumps a year ago, otherwise been fairly healthy but not robust, frequently troubled with swollen tonsils.	Good	Negative.

3. G. Family.

	Age	Infective History.	Present Health	Agglutinating Reaction
Mr G.	33	Had measles, hooping cough and chicken pox in infancy. Has had 6 attacks of Rheumatic Fever, the first when 7 years old and the last a year ago.	Good. Heart sounds clear and action regular.	Positive.
Mrs G.	36	Had measles and hooping cough in childhood and been occasionally troubled with joint pains.	Good	Positive.
Isabella G.	18	Had measles and hooping cough in childhood.	Good	Positive.
Jessie G.	9	Measles and hooping cough in childhood.	Good	Positive.

4. A. Family.

	Age	Infective History.	Present Health.	Agglutinating Reaction
Mr A.	65	Measles, Scarlatina and hooping cough in infancy; small pox when 33 years of age; slight and occasional rheumatic pains and influenza twice.	Good	Negative.
Mrs A.	59	Measles in childhood; much troubled with rheumatic pains all her days; and influenza several times.	Good	Negative.
Mr John A.	27	Had measles and Rheumatic Fever, the latter 13 years ago, no complications nor recurrences.	Good	Negative.

5. C. Family.

	Age.	Infective History.	Present Health.	Agglutinating reaction
Mr C.	36	Measles and hooping cough in childhood. No Typhoid or Rheumatic Fever. Was delicate till 12 years old and healthy since.	Good.	Positive.
Mrs C.	30	Had measles, Scarlatina (twice) hooping cough and chicken pox. Has always been strong except for some uterine trouble (displacement) after birth of first child.	Good.	Negative.
Christina C.	6	Has had Hooping cough otherwise been well.	Good.	Negative.

6. L. Family.

	Age	Infective History.	Present Health.	Agglutinating Reaction
Mr L.	60	Hooping cough in infancy, no measles, Scarlatina nor Rheumatic Fever, but influenza two or three times.	Good	Positive.
Mrs L.	52	Diphtheria, measles twice and influenza several times, no rheumatism or Scarlatina.	Good	Positive.
Margaret L.	16	Measles, hooping cough and chicken pox. No Scarlatina nor rheumatism.	Good	Positive.
Robert L.	13	Measles, hooping cough and chicken pox - also a feverish gastro-intestinal attack (10 days) last year. No Scarlatina nor Rheumatism.	Good	Positive.

I regret that the cases are so few, but such are the limitations of research work in general practice. I was amazed to find how few people (even well educated people) were willing to be victims of science even to the extent of having their fingers pricked, when it was "not to do them any good."

So far as it went, however, the results did not give grounds for any definite generalisations of a positive nature.

Of the twenty individuals, all healthy, the sera of thirteen agglutinated my washed reds and seven did not.

An extensive infective history did not always result, so to speak, in a positive reaction, nor did a meagre infective history insure a negative one.

As regards the congenital or hereditary question, if both parents were positive, the offspring were all positive; if both parents were negative, the offspring were negative.

If one parent was positive and one negative the offspring was negative.

But as I said before the cases were far too few to found any generalisations thereon.

So far my results were inconclusive and even contradictory and in the light of subsequent knowledge inevitably so.

The two main errors or ignorances contributing to this unsatisfactory result were:-

1. The assumption that the phenomenon of isoagglutination was something abnormal and therefore indicative of disease past or present.
2. The assumption that any individual's blood could be taken as a standard of health if his blood serum did not agglutinate the washed red corpuscles of another individual or even of two or three other individuals taken at random.

As regards the first assumption I found on subsequent reading that it had been demonstrated years ago by Landsteiner,[2] Descatello and Sturli,[3] Shattock[4] and others, that considerably over 90% of normal bloods (i.e. the blood of healthy individuals) can shew this phenomenon of isoagglutination and that it cannot therefore be regarded as a manifestation of disease.

As regards the second assumption - the fact that the phenomenon is dependent on two factors, (a) active serum and (b) susceptible corpuscles, is apparently lost sight of.

A given serum tested against the washed reds of two or three other bloods may give no reaction because those bloods contain no corpuscles susceptible to the action of the haemoagglutinin contained in that serum; and yet that serum might find susceptible corpuscles in some

other blood. Vice versa corpuscles not agglutinated by one serum may be agglutinated by another. And such indeed is found to be the case if one takes a sufficient number of bloods, prepares serum and washed reds from each, and tests each serum against each specimen of washed reds in series.

This has been done by several observers. Amongst others, Descatello and Sturli, o.c. in a series of 155 cases found only four whose serum had no agglutinating effect on the washed reds of any of the others.

In the Practitioner of May 1908 I found an article by Fleming[5] on some sources of error in the estimation of opsonic indices. In the course of the article he referred to this phenomenon of isoagglutination, gave some observations of his own, and referred to recent work on the same subject by Hektoen of Chicago.[6] The latter besides giving a very complete resumé of the subject and some original observations of his own, tabulated a number of references to continental authors, chiefly German. Many of these I have studied and now propose to give the results of that study under three or four different headings.

I. Historical.

The great discovery of Gruber[7] in 1896, in which he demonstrated the agglutination of typhoid bacilli by the serum of a typhoid patient, gave rise to much experimenting and research on the subject of agglutination generally.

It was soon shewn that this specific agglutination could be produced not only against bacteria but also against various other cellular elements. Bordet[8] for example, by injecting one animal with the blood of another of a different species, was able to produce an agglutinin for the red cells of the latter. The serum of the immunised animal agglutinated the red cells of the animal with whose blood it had been injected. And even with animals of the same species - Ehrlich and Morgenroth[9] by repeatedly injecting a he-goat with the blood of a she-goat, ultimately succeeded in bringing about that the serum of the former agglutinated the red cells of the latter, and also the red cells of other she-goats.

This specific immunity (if it may be so called) against various other cellular elements was produced e.g. by Landsteiner[10] against spermatozoa, by Von Dungern[11] against ciliated epithelium, and by Metschnikoff[12] against leucocytes, the serum of the animal injected developing a special agglutinating power for the variety of cell injected.

But besides this specific immunity or induced agglutinin it has been repeatedly shewn that normal serum possesses an agglutinating power over various bacteria. Cholera bacilli are vigorously agglutinated by normal serum (Bordet).

Halban[13] shewed that normal serum could produce

agglutination phenomena with spores of bacillus subtilis, and Kraus and Low[14] demonstrated suspension of molecular movement of other bacteria under the influence of normal serum. Lastly, as normal human serum has the power of agglutinating bacteria, so also has it the power of agglutinating normal human erythrocytes.

This isoagglutination, being dependent on two factors (agglutinating power of serum and agglutinability of corpuscles) and those two factors moreover being both subject to variation in quantity and in quality, it follows that classification must be difficult, if not impossible.

II. Classification of Human Bloods as regards Isoagglutination in Health.

Landsteiner (o.c.) as the result of an examination of a series of specimens, preparing serum and washed corpuscles from each individual, and testing each serum against each specimen of corpuscles, evolved a classification which has proved wonderfully comprehensive and constant, as evidenced by the unanimity with which it has been confirmed and adopted by many subsequent observers. He found that individuals might be divided into three main groups.

In Group I. are included those whose sera agglutinate the corpuscles of Groups II and III and whose corpuscles are not agglutinated by any sera.

In Group II are included those whose sera agglutinate the corpuscles of Group III and whose corpuscles are agglutinated by the sera of Group III (and I.)

In Group III are included those whose sera agglutinate the corpuscles of Group II and whose corpuscles are agglutinated by the sera of Group II (and I.)

In Group III are apparently also included bloods whose corpuscles are agglutinated by the sera of Groups I and II but whose sera possess no agglutinating power.

There are two obvious objections to this grouping. Firstly - Groups II and III are on the same plane; they have the same relation to Group I, and their relation to each other is reciprocal and equal, the corpuscles of each being agglutinated by the sera of the other, and the corpuscles of both being agglutinated by the sera of Group I.

To put it in another way there is nothing to determine which should be labelled II and which III. As a matter of fact, in the investigation and classification of a series of bloods, apparently that group of the two which has the greater number of examples is labelled II, and the other III.

A second objection is the placing in Group III of bloods which shew no agglutinin and cannot therefore fit the definition of Group III as to agglutinating the corpuscles of Group II.

I venture therefore to suggest another classification.

Group I to include those bloods whose corpuscles are not agglutinated by any sera and whose sera agglutinate the corpuscles of all the other Groups.

Group III at the other end, so to speak, to include those bloods whose sera do not agglutinate the corpuscles of any of the groups and whose corpuscles are agglutinated by the sera of all the other groups.

Group II, an intermediate one, (with subdivisions) whose sera agglutinate the corpuscles of Group III but not those of Group I, and whose corpuscles are agglutinated by the sera of Group I but not by those of Group III; the subdivisions of this group moreover shewing interagglutination, the sera of each agglutinating the corpuscles of the other. The subdivisions might be named IIa and IIb.

Or again these Groups I, II, III might be named respectively "The Non-agglutinable Group", "The Inter-agglutinating Group" and the "Non-agglutinating Group."

Annexed I place three Tables.

Table II is taken from Hektoen's article above referred to. It has been rearranged to show the grouping into Landsteiner's three classes.

Table I, I have worked out backwards from this, shewing how the results would come out before any rearrangement. Glancing one's eye down the columns one can easily pick out the three groups.

Table III shews the same results rearranged according to the grouping which I have suggested above.

Table I.

Corpuscles

Sera

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
4	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
5	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
12	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
13	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
14	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
15	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−	−	+	+	−	+
16	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
19	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+
20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−

Table II

Corpuscles

Sera

		Group I.							Group II											Group III	
		4	5	12	13	14	16	19	1	2	3	6	7	8	9	10	11	18	20	15	17
Group I	4	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	13	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	16	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Group II	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Group III	15	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	⊕
	17	−	−	−	−	−	−	−	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	−	−

Exceptions to Landsteiner's Grouping indicated by enclosure in circles ○

Table III

Corpuscles

Sera

		Group I							Group II a											Group II b	III
		4	5	12	13	14	16	19	1	2	3	6	7	8	9	10	11	18	20	15	17
Group I	4	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	13	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	16	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Group II a	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	18	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Group II b	15	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+
" III	17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

The Constancy of the Grouping.

To shew the constancy of the grouping and to eliminate the possibility of its being merely fortuitous, Hektoen repeated his experiments on more than one occasion, and at intervals of a month or two, using the same individuals, all apparently healthy young adults. With one or two slight exceptions, the results were identical on all occasions. The exceptions were that on one of the three occasions a serum of Group I failed to agglutinate all the corpuscles in Group II, and also on one of the three occasions a serum of Group II shewed no agglutinin for the corpuscles of Group III.

To confirm this constancy still further the same observer took blood from five healthy individuals so chosen as to furnish examples of each of Landsteiner's three groups. These he tested against each other "at frequent intervals throughout a period covering several months." The results were always the same with one exception viz. the blood of one of the individuals in Group III gradually lost its agglutinin.

So much for the grouping as regards isoagglutination in normal bloods.

Classification as regards Isoagglutination in Disease.

The phenomenon has also been investigated in series of patients suffering from various diseases, and all the observers agree in stating that the same grouping

occurs and in fairly similar proportions. Even taking a series of cases of the same disease, Descatello and Sturli (op. cit.) found representatives of all three groups.

Average percentage in the different Groups.

The relative numbers in the different groups of course vary somewhat in different series of cases and it is difficult to get a sufficient number of cases from which to infer what might be called an average.

Fleming examining 138 bloods placed 46 in Group I and 92 in Groups II and III.

Hektoen with 76 cases (healthy and diseased) had 36 in Group I and 40 in Groups II and III.

Descatello and Sturli in 34 healthy adults had 19 in Group I, and 15 in Groups II and III; in 121 diseased individuals they found 47 in Group I and 74 in Groups II and III.

Putting these together we have 369 cases with 148 in Group I and 221 in Groups II and III or approximately 40% in Group I and 60% in Groups II and III.

In striking average percentages for Groups II and III separately or for Groups IIa, IIb, and III (of my proposed classification), Fleming's figures are not available but the other two sets are:-

Hektoen's 76 cases gave 26 in IIa, 8 in IIb, and 6 in III.

Descatello and Sturli's 34 healthy cases gave 10, 4 and 1 respectively. Descatello and Sturli's 121

diseased cases gave 48, 23 and 3 respectively.

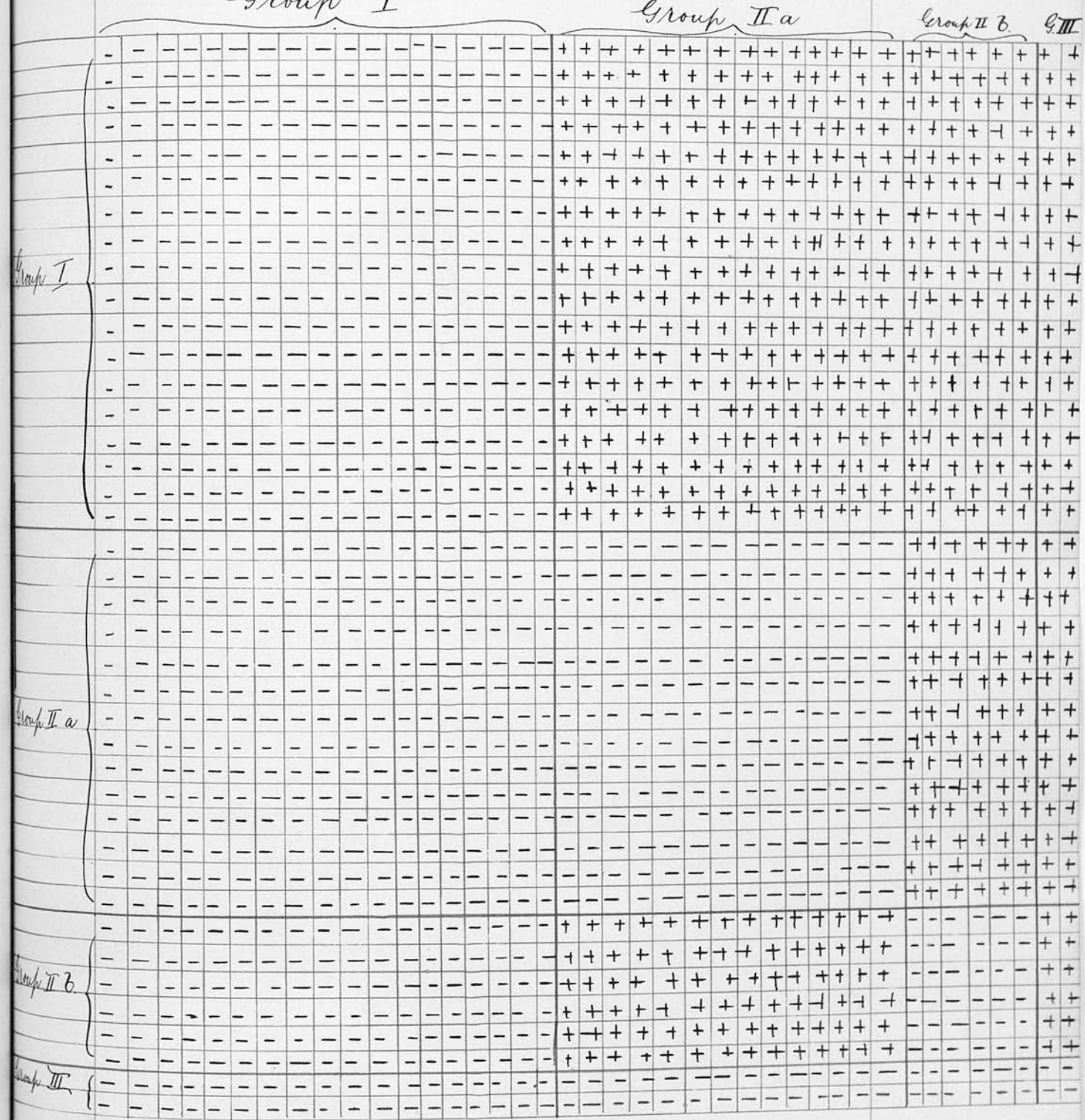
Putting these together we have 231 cases with 84 in Group IIa, 35 in Group IIb, and 10 in Group III, or percentages approximately of 36%, 15% and 4%, leaving 45% for Group I as against 40% when Fleming's cases were included.

Speaking roughly and approximately and in figures readily carried in the memory, I think we may say that in a long series of bloods examined with respect to isoagglutination, we might expect to find 40 - 45% in Group I, about 35% in Group IIa, about 15% in Group IIb, and about 5% in Group III. (See Table IV.)

Table IV

Corpuscles

average distribution in 40 bloods



Group I 45 % = 18

" II a 35 % = 14

" II b. 15 % = 6

" III 5 % = 2

40

The Number of Haemoagglutinins or Isoagglutinins.

It is evident from the above grouping that there must be more than one agglutinin commonly present in human blood separately or together. Mere difference of strength or quantity of a single agglutinin could not permit of the above classification. The corpuscles must be immune (so to speak) to an agglutinin present in the serum in which they have been accustomed to float. Consequently agglutination never occurs between sera and corpuscles of the same group.[2]

It would be equally impossible to imagine agglutination between sera of Group IIa and corpuscles of Group IIb and at the same time agglutination between the sera of Group IIb and corpuscles of Group IIa (Interagglutination) if there were only one agglutinin.

Footnote 2.

Auto-agglutination has been described by some observers.

Fleming[5] says it is very common in patients in hospital especially in anaemic persons and in those suffering from certain acute diseases notably Pneumonia, Erysipelas and Acute Rheumatism.

Shattock[4] also says this auto-agglutination so called is an invariable phenomenon in the clotting of horse's blood where the appearance described as the buffy coat constantly presents itself. The upper part of the clot, the buffy coat, contains serum fibrin and entangled leucocytes, the red corpuscles sedimenting and exhibiting long rouleaux formation.

Fleming moreover states that in ordinary clotting in vitro if the coagulative time of the individual be lengthened and especially if the person be anaemic this formation of buffy coat and sedimenting of the erythrocytes with rouleaux formation can frequently be observed.

Lastly Fleming affirms that even in cases in which he found this auto-agglutination, if serum and washed corpuscles were prepared from the patient separately and then brought together no agglutination followed. He concludes that the one is perhaps a weaker form of the other.

(Continued.)

We must postulate at least two Haemoagglutinins.

Hektoen holds that there are three at least, one for each of Landsteiner's three groups and he thinks there may be others.

Descatello and Sturli, Landsteiner agreeing, only postulate two distinct and commonly present agglutinins, and on theoretical grounds only two appear to be necessary to fit in with the above classification.

An agglutinin (a) present in sera of Group IIa, an agglutinin (b) present in sera of Group IIb, both present in sera of Group I, and both absent in sera of Group III, would on theoretical grounds at least give a consistent basis for, and explanation of, the above mentioned classification.

This theory Descatello and Sturli put to the test of absorption experiments in various combinations as follows.

Footnote 2. (Continued)

It seems to me that they are totally different and distinct. The one is simply a delayed coagulation (habitual in horse's blood) permitting sedimentation of red cells and rouleaux formation.

The other is a specific reaction without the presence of fibrin which I believe is an essential element in ordinary coagulation in vitro.

Rouleaux formation also, I believe, never takes place in the absence of fibrin, and is never seen so far as I know in the phenomenon of agglutination as above described.

Anyhow I think it may be affirmed that auto-agglutination in the sense of agglutination between the serum and washed red corpuscles or even between the serum and red corpuscles in the defibrinated blood of the same individual never occurs either in health or disease.

(End of Footnote).

Mixing thoroughly a serum of Group IIa with an excess of washed corpuscles from a member of Group IIb and centrifuging the mixture they found that the serum had lost its agglutinating power not only for the corpuscles of that member but also for the corpuscles of any member of Group IIb. The reverse experiment also held good - a serum of Group IIb exhausted of its agglutinin by excess corpuscles of a member of Group IIa shewed no agglutinin for corpuscles of any other member of Group IIa. But if they mixed a serum of Group I with excess corpuscles of Group IIa, they found that the serum had lost its agglutinating power for corpuscles of any member of Group IIa, but that it was still active for corpuscles of any member of Group IIb; and so also exhausting serum of Group I with corpuscles of Group IIb, still left it active against corpuscles of Group IIa. This seems to shew conclusively that there are only 2 commonly occurring isoagglutinins in normal blood, appearing sometimes separately (Groups IIa and IIb) and sometimes together or combined (Group I.)

The amount of agglutinin varies even in members of the same group and so also does the susceptibility of corpuscles in the same group.

All the observers mentioned are agreed as to this, and it is easily shewn in the usual way by dilution experiments. For example Fleming found that of two sera

from the same group one agglutinated even when diluted 64 times while the other failed when diluted 4 times although the undiluted serum had, in both cases, given marked agglutination.

So also of washed corpuscles from two individuals of the same group, both susceptible to a given serum when undiluted, the one might still prove susceptible to that serum when diluted many times, whilst the other proved refractory to the mildest dilution of the serum, giving no reaction.

I have already indicated that there appears to be no difference between healthy and diseased conditions as to the presence of isoagglutinins or even as to their distribution and grouping, nor according to Hektoen does there appear to be a larger amount of these substances, as determined by dilution experiments, in the blood of patients suffering from scarlatina, pneumonia, typhoid and tuberculosis than in the blood of healthy persons.

But the interesting question remains whether disease produces an alteration in the quantity or quality of these substances or in the susceptibility of the red cells, and if so, in what direction or to what extent.

Investigation of this question by experiment in the human subject should be to some extent possible in this age of vaccinations and inoculations.

Amongst the lower animals isoagglutinins do not seem to occur naturally. Hektoen made an extensive investigation of this point amongst rabbits, guinea pigs, dogs, horses and cattle. In each class 10 or 20 different sera were tested against as many samples of washed corpuscles from the same species without once producing agglutination.

Bruce o.c. succeeded in producing the phenomenon of isoagglutination in rabbits[x] by inoculating them with pathogenic organisms but the reaction in that case might be due to specific agglutinins which are doubtless closely related bodies and possibly even modifications of the same substance, but they are not the same. For example one cannot imagine specific agglutinins (so called) in the blood of new born infants of healthy mothers but isoagglutinins are certainly present in the blood of a considerable proportion of such infants according to Halban.[15]

I shall return to his observations later in discussing the nature and origin of isoagglutinins.

The mechanism of agglutination has been the subject of much research and discussion and of many conflicting views and theories. The two chief lines, however, are well marked and may be called the inorganic and the organic, or the physical and vital, - the former including mechanical, chemical and electrical agencies

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x So also Bordet and Ehrlich and Morgenroth by injecting blood vid. sup.

or forces. Some observers lean more to the one class of forces to explain the phenomenon and other observers lean more to the other class.

Kraus and Löw have shewn that agglutination phenomena can be produced with substances and media entirely inorganic and inert in themselves. They indicate that if a fine wash of Indian ink or of ultramarine or cinnabar be placed in the well of a hanging drop slide and examined under the microscope there is observed a lively molecular movement of the particles (Brownian movement). On the addition of a few drops of absolute alcohol, however, an immediate change takes place - the Brownian movement ceases and the particles of pigment run together into heaps or clumps in an exactly similar manner to that of bacilli under the influence of an immune serum or of red corpuscles under the influence of isoagglutinin.

With micro-organisms also, other observers, Blackstone, Engels, etc., have shewn that agglutination can be effected by inorganic chemical substances, such as acids, alkalies, saffronin, etc.

These experiments were made both with living and dead micro-organisms, though doubtless the living were immediately converted by the action of the chemicals into dead organisms, and then acted or were acted upon exactly as the inert particles of pigment in Kraus and Löw's experiments, obedient to the forces of

molecular attraction and repulsion. These forces may be of the nature of chemical affinities or dependent on electrical properties (positive and negative) and subsisting not only between the particles themselves, but also between the particles and the molecules of their surrounding medium.

As regards the agglutination phenomenon between organised agglutinins and organic material such as bacilli or erythrocytes and in an organic medium such as blood serum one would naturally expect that a satisfactory theoretical explanation could not be furnished solely by mechanical or chemical laws. Bordet op.cit. after a lengthy and learned discussion of the various theories advanced, comes to a conclusion which is of the nature of a compromise or compound of the mechanical and the vital points of view.

He divides the phenomenon into two stages and even says that the first stage can be produced without provoking the second. The first stage consists in the union of the agglutinin with the agglutinable cell, a living, active, and vital process dependent on a living, active, and vital affinity between living things.

The result is death of the cell and its deprivation of the subtle vital properties by which it maintains its independent and separate equilibrium in the surrounding medium. It is, henceforth, entirely a passive agent, it may be with swollen and viscous cell

membranes, and entirely at the mercy of the changed molecular attractions between itself and its dead neighbours and between them and the surrounding medium.

The second stage, the agglomeration or clumping Bordet regards as entirely mechanical, the result of physical forces acting on inert material, and essentially similar to the clumping of the pigment particles in Kraus and Löw's experiment.

The Nature and Origin of Isoagglutinins.

In their chemical constitution isoagglutinins, like specific agglutinins, appear to belong to the class of globulins, being precipitated by saturated ammonio sulphate solutions, and capable of recovery from the filtered residue by redissolving with normal saline. Descatello and Sturli put this to the proof with serum from examples of each of Landsteiner's three groups and found that after precipitation and redissolving as above, the agglutinin was apparently unaltered in its agglutinating reactions, remaining true to the group from which it had been taken.

The origin, source, derivation or evolution of agglutinins generally, and of isoagglutinins in particular, is a difficult and complicated problem and, as far as I can make out, still far from solution.

Having been first discovered in connection with the investigation of diseased conditions, the tendency

was to regard them as extrinsic, foreign or imported substances, only present in disease and totally disappearing sooner or later on convalescence.

Further observation and experiment however, soon revealed the fact that normal serum contained agglutinin for many pathogenic microbes.

Kraus and Löw for example, in the communications above referred to, made an extensive investigation on this subject on comparative lines and found great differences in the agglutinating power of normal sera to various microbes, the differences being marked not only between the sera of different species, but likewise between different individuals of the same species to the same microbe.

Some of their findings are of great general interest - normal blood serum of man and of mammals almost always agglutinated bacillus coli and also staphylococci but not streptococci, Friedlander's bacillus, or cholera vibrios. (This last is contrary to Bordet's observations quoted above.)

These observers further state that "the normal serum of men frequently agglutinated bacillus coli in dilutions of 1 in 50 and staphylococci in 2 hours at most, in dilutions of 1 in 100."

The question naturally arises whether this property of normal serum is hereditary and congenital or acquired.

In the case of human serum it is easy to conceive

a widely prevalent acquired immunity for both bacillus coli and for staphylococci, the former of these organisms being a normal inhabitant of the human intestine, and the latter also normally present on skin and mucous membranes.

An auto-immunisation is, therefore, both possible and probable sometime during the life of the individual, so also in the case of the lower animals.

As to whether such specific immunities are ever hereditary or congenital in the human subject, I find no reference in the writings I have consulted. But, as bearing on this point, Kraus and Löw state that the serum of young guinea pigs in their experiments never agglutinated bacillus coli whereas in healthy adult guinea pigs the agglutination was constantly present and often in dilutions of 1 in 10 or even 1 in 20.

It would not be very wonderful with fuller knowledge to find a correspondence between the agglutinins in the blood of different species of animals and the microbes commonly present in their parasitic or epizootic flora - such of them at least as are capable of invading the tissues of their host, and infecting the blood or lymph streams.

As distinguished from agglutinins generally, the origin and source of haemo- or iso-agglutinins remains to be considered.

Halban[15] with a view to throwing light on this

point, made a series of observations on iso-agglutination in foetal and maternal blood. The foetal blood he took from the umbilical cord of new-born infants and the maternal blood from the post partum uterus as it flowed therefrom during a period of relaxation. Of each he took two test tubes full, the one for centrifuging to obtain serum, and the other for defibrinating. The defibrinated blood he evidently considers equivalent (for the purposes of this reaction) to washed red blood corpuscles, the defibrinated serum being equivalent to isotonic salt solution.

Whether they be strictly comparable or not, defibrination would probably exclude ordinary coagulation or rouleaux formation, phenomena which I believe only take place in the presence of fibrin.

He tested the foetal and maternal sera against the foetal and maternal corpuscles in various combinations, with the following results:-

1. The foetal and maternal blood from infant and its own mother, acted like bloods from two different individuals.
2. The maternal serum in some cases agglutinated the corpuscles of her own infant and inversely the serum of the infant in some cases agglutinated the corpuscles of its own mother.
3. On the whole the maternal serum appeared to agglutinate more frequently and more strongly than the foetal sera; and as a corollary the foetal cor-

puscles were generally more susceptible and less resistant to agglutination than the maternal corpuscles.

With these results before him, Halban proceeds to discuss various theories of origin of isoagglutinins -

1. That they may result from a mutual immunisation between mother and infant he considers refuted by the fact that his experiments shew that mother and infant are, in many cases, not mutually immune as regards isoagglutination.
2. That they may result from the absorption of various bacterial products, chiefly intestinal, he dismisses on the ground that, as he has shown, they are already present in the blood of new born infants, "whose bodies never contain bacteria."

He adds as a footnote, however, that "Kraus and Claremont have recently shown that the serum of newly born doves possesses strong bacteria-dissolving properties."

I would here note also that Rosenberger[16] has recently demonstrated tubercle bacilli in the blood from the umbilical cord of infants born of tuberculous mothers.

3. The third theory that Halban discusses is that which attributes the formation of haemoagglutinins to a sort of self-immunisation from the continual destruction of red blood corpuscles in the spleen and elsewhere a constant process in the living human body, both adult and foetal.

He seems to consider that this theory requires the confirmation of experiment and that such confirmation would be afforded if the injection into an individual of his own blood resulted in an increase of his haemoagglutinins.

He adds that the one experiment of this sort recorded by Ehrlich and Morgenroth, gave a negative result.

Halban finally comes to the conclusion that the agglutinating power of normal serum is a congenital property of the substances normally in the blood. Before formulating my own conclusions as to the origin or evolution of agglutinins and their significance, - conclusions arrived at by comparing and contrasting and pondering over the views of the several workers in this field, I should like to make one or two very general preliminary observations which have occurred to me in the course of this study.

I. The first of these preliminary general observations is that agglutinins of any kind have never, so far as I know, been isolated or identified, and yet we hear them constantly spoken of as if they were substantial and separate entities. Their functions we know partly, and can demonstrate them even to the naked eye; their associations we know; their intimate relations with the globulins of normal serum is a proved and

accepted fact; but their separate existences are merely hypothetical and inferred from their visible functions.

For anything we know to the contrary, globulins or a constituent part of globulins, may be the physical basis of the hypothetical substances called agglutinins. Further this primary physical basis may be capable of development by re-arrangement of its molecules or by the accretion of new molecules (pendants I think they are sometimes called) to subserve the functions of many different agglutinins. Such re-arrangements or accretions may be temporary or permanent in various degrees, according to the capacities or necessities of the organism.

A theory of this nature may be exemplified or illustrated by the analogy of an army or body of troops in action. They may simply change their front or change their formation by re-arrangement to meet different kinds of dangers, or dangers coming from new and different directions; they may call up reserves to join the body in action (accretion) to meet other and added dangers.

To return to our primary agglutinating substance, it may not be necessary to postulate even re-arrangement or accretion to meet new dangers but merely a change of front.

It may be many sided and capable of acting in

different directions with different activities. Interesting analogies to this are furnished by nature in other fields and even in the inorganic world. For example the apparently single and simple substance radium, has been shewn to give off three different kinds of rays called the Alpha, the Beta, and the Gamma rays, each with different and well marked characteristics, and there may be other sides and other rays not yet discovered.

So also our "primary agglutinating substance" may not only have two or three sides, but may be capable of developing other sides (with other agglutinating activities) under suitable stimulus.

Theoretically at least, we can place no limitation to the potentialities of evolution and development in living things.

II. My second general observation relates to the definition of various terms used in connection with the investigation of agglutinins or agglutinating substances - such as "natural", "normal", "congenital", "acquired", "specific".

It appears to have been generally taken for granted till recently that the ordinary pathogenic microbes (excluding zymotics and syphilis) are never transmitted from mother to infant. Rosenberger has recently added tubercle bacilli to the list of ex-

ceptions, and other disease germs may have to follow, in the rapidly increasing light shed by bacteriology. But on this questionable assumption has been founded another no less questionable, namely that the blood of the new born infant can contain no agglutinins and especially no "specific" agglutinins - that these can never therefore be hereditary or "congenital" but always "acquired" after birth.

Further a very general assumption seems to me prevalent that "normal" serum - serum from the blood of healthy adults - should never contain "specific" agglutinins, as such can only be "acquired" as the result of invasion by disease germs. This would make out nature to be but a very indifferent general, making no preparations for defence or protection till invasion had taken place.

Defensive and protective forces agglutinins certainly are, the first stage in the agglutination phenomenon being the killing or crippling (or depriving of its vital properties) of the substance to be agglutinated, be it foreign cell, or microbe (vide supra under mechanism of agglutination.)

Moreover, we have already seen that nature does make such protective provisions without the intervention of disease and long before an invasion. By a process of self immunisation she enables a normal healthy adult to lay up stores of "specific" agglutinin for bacillus coli (or to develop powers of agglutin-

ating b. coli) to such an extent that if his serum be tested it is found to agglutinate that organism even when diluted 50 times - a good provision this against invasion!

Conclusions as to origin and evolution of Agglutinins in General.

I believe that Nature is still more foreseeing and farsighted - that she does not wait for the birth of a child (or an animal) before commencing her protective work. I believe she begins at the beginning - in utero - and ordains that the individual shall be endowed by its parent with a protective substance - doubtless one amongst many such-but this one specially useful against microbes and perhaps against some other "organised foreign bodies" potentially noxious to the individual.

This protective substance we shall call "primary agglutinating substance". It is primary only with respect to the individual inheriting it. It is already stamped with potentialities inherited and transmitted by a long line of countless ancestors and during the lapse of countless ages.

It may have been stamped with other potentialities in previous ancestors and ages and gradually lost them from changed environment and absence of their causative stimuli.

It may on the other hand in the individual now

inheriting it or in his descendants through the ages gradually acquire (and transmit) new potentialities brought into being by new stimuli in new environments. During the life of the individual some of the potentialities may remain dormant and latent from not coming across their appropriate stimuli - others may from meeting their appropriate stimuli in great strength be called upon for very active functioning. If equal to the strain they will be strengthened and gradually through successive generations transmitted in greater strength; if unequal to the strain the result will be death of the individual; but other individuals of the same species and generation with that special potentiality more strongly developed may prove equal to the strain, and surviving, hand on the potentiality in greater strenth.

In short, to my mind, specific agglutinins are not new substances but new developments - the calling out and realizing in function of potentialities previously dormant or latent but nevertheless inherent in the primary agglutinating substance which is inherited and congenital. I do not put forward this view or the preceding parable as in any way original.

It is the result of comparing and contrasting the views of several authors and especially thinking over one illuminating sentence of Bordet's which perhaps sums up the matter even better and certainly more tersely.

He says op.cit. "The special properties of immune sera exist in germ or potentiality in normal sera."

There is a third general observation with a practical bearing which I wish to bring forward.

In the phenomenon of isoagglutination as well as in that of specific agglutination, there are always two factors concerned, and one of them is apt to be lost sight of. For example if a given serum fails to agglutinate the washed blood corpuscles from 20 or 30 other individuals it is said to have no agglutinin. I doubt if this is ever strictly true. If, instead of testing it against 20 or 30 we tested it against 200 or 300, we might come across a sample of red cells which it did agglutinate; and even if we did not, that serum might nevertheless contain agglutinin, but in quantity or quality too weak to overcome the resistance of the corpuscles against which it had been tested. For purposes of classification it is useful and necessary to group such sera in a class by themselves, but it would be more accurate and scientific to describe the class as containing sera which shew no demonstrable iso-agglutinin.

So also with specific agglutinins the agglutination phenomenon depends for its manifestation on a suitable relation between the two factors concerned, viz. the agglutinating strength of the agglutinin and

the resisting strength of the microbes, and both may vary between wide limits.

The reaction may fail to shew itself in a given case either from weakness of the agglutinin or from strength of the microbes. But agglutinin and even specific agglutinin may nevertheless, be present, and with weaker specimens of the same microbe might manifest itself.

One would expect agglutinins to be weaker and less developed in a child than in an adult, and weaker and less developed in an adult who had had few illnesses than in an adult who had come through several illnesses and had his agglutinins developed by exercise in combating various microbic onslaughts.

On the other hand, microbes of the same species must vary greatly in different samples as regards their strength and virility. virulence

It is well known that there are many strains, as they are called, of the same micro-organism, varying greatly in their activity and virulence. Also it is well recognised that the age of a given culture of a pathogenic microbe undoubtedly modifies its virulence and presumably also its resistance to agglutination.

From these considerations I have two deductions to make -

Firstly, before agglutination phenomena can be of much use in diagnosis or prognosis it is absolutely

essential that there be devised some reliable method of standardising both factors to the reaction. "Normal serum so many times diluted" can be no reliable standard in presence of the fact that normal sera differ considerably amongst themselves in agglutinating power.

So also a "fresh culture so many hours or days old" of a given microbe can be no reliable standard of resistance in light of the fact that there exist many strains of the same microbe with different degrees of virulence and presumably of resistance to agglutination.

Secondly it cannot be absolutely affirmed and proved of any blood serum that it contains no agglutinin or iso-agglutinin. All that can be truthfully stated is that it contains none demonstrable, or that it contains none in relation to some particular standard.

Such relative standards are, of course, both legitimate and useful for classification purposes, but it must be constantly kept in mind that they are relative, not absolute. Indeed in an enquiry of this kind one is being constantly impressed with the difficulty and futility of attempting to confine the infinite variety of nature within the hard and fast lines of any classifications. They are, at the best, always relative and continually demanding modifications, exceptions, and other forms of compromise.

The Nature and Significance of Iso-agglutinins.

The agglutination of red cells is probably effected by the same substance as agglutinates bacteria; or, to put it in another way, I think it likely that further research will show that iso-agglutination and specific agglutination are different functions of the same constituent of the blood. I feel confident also that further investigation will show that this primary agglutinating substance is present in all bloods of all animals, but in varying quantities and strengths and with varying functional developments - varying in these respects, not only as between animals of different species, but also between animals of the same species.

The normal serum of some animals agglutinates the red corpuscles of some other animals of a different species, - a phenomenon named hetero-iso-agglutination to distinguish it from idio-iso-agglutination, the name bestowed upon the same phenomenon between individuals of the same species.

Further this hetero-iso-agglutination, even when it does not exist normally between two species, can sometimes be induced by injecting the blood of one species into an individual of the other species - an induced hetero-iso-agglutination. But the curious thing is that apparently in the human species only, does idio-iso-agglutination occur naturally, though it has been induced in goats by injecting the blood of one goat into another goat (vide supra).

Why this should be so is an interesting question, but possibly no more capable of explanation or solution than an inquiry as to why red corpuscles should be round in one species and oval in another.

The diagnostic value of Iso-agglutination is at present nil, and will continue to be nil till such time as further research may yield a knowledge of the exact causation and significance of the phenomenon, and its relation to the phenomena of specific agglutination.

That there is a close relation between the two Bruce (op.cit.) has shewn by the experiments in which he induced the phenomenon in rabbits by injecting them with pathogenic microbes.

A practical point, however, in connection with the phenomenon is its occurrence in estimations of opsonic indices.

Fleming (op.cit.) has worked out carefully the question of its influence in this connection and comes to the conclusion that the presence of agglutinable corpuscles in the corpuscular mixture has a marked effect on the opsonic index and always on the side of increase. That is to say, that the same serum gave a higher opsonic index when agglutinable corpuscles were used than when no such corpuscles were present in the corpuscular mixture.

He advises therefore that by previous testing

such agglutinable corpuscles should be excluded, and that, generally speaking, only corpuscles of bloods belonging to Landsteiner's Group I, should be used in the estimation of opsonic indices.

SUMMARY of CONCLUSIONS.

A. As Regards Agglutination Generally.

1. The phenomenanof agglutination may be a purely mechanical one, and can be shewn with inert particles in an inorganic medium and with an inorganic determining cause.

2. With organic materials the phenomenon is divisible into two phases - a vital and a mechanical. The vital phase results in the death of the resisting factor and its subsequent amenability to mechanical or physical laws.

3. All bloods probably contain an agglutinating substance intimately related to or incorporated with the globulin constituent of the serum. This agglutinating substance occurs in varying quantity in different individuals and in different species. It is capable of various functions and doubtless endowed with the potentiality of developing new functions.

4. In the study of agglutination generally, the fact of its dependence on the inter-relation of two factors must never be lost sight of.

5. The scientific use of this phenomenon for purposes of diagnosis or treatment, depends on the possibility of devising methods of standardising each of the two factors.

6. A third factor has also to be remembered viz. the medium - its constitution, temperature etc. - whether it be normal saline, or distilled water - whether the conditions be in vitro or in corpore etc.etc.

B. As regards Isoagglutination.

1. Isoagglutination occurs naturally only in the human species, though it can be induced in other animals.

2. There appear to be two distinct isoagglutinins, commonly present in the blood of human beings, healthy and diseased, sometimes occurring separately and sometimes combined, co-existent, or co-ordinated. One of these two, haemo-agglutinins, is apparently of more frequent appearance than the other.

3. The resulting classification is naturally into 4 groups - 1, those containing both agglutinins, 2, those containing only the more common of the two, 3, those containing only the less common of the two, and 4, those containing no demonstrable haemoagglutinin.

4. The average frequency of these groups in a long series of cases appears to be about 45% in Group I, 35% in Group IIa, 15% in Group IIb, and 5% in Group III.

5. The grouping appears to be fairly constant in different series of bloods and in the same series when re-examined on many occasions at considerable intervals of time.

6. The grouping appears to be nowise different in diseased conditions as compared with healthy ones.

7. The amount of haemoagglutinin in different members of the same group varies considerably as shown by dilution experiments.

8. Haemoagglutinins and specific agglutinins appear to be similar and related bodies or substances and their functions may be interchangeable. They are thermostable - heat does not alter their specific character, though it does weaken their action as shown by dilution experiments.

9. Agglutination and Coagulation appear to be separate and distinct phenomena, though they may have obscure and subtle relations which have not been worked out.

10. Auto-agglutination, as between serum and washed reds or between serum and defibrinated blood from the same individual and prepared separately, never occurs.

11. The diagnostic value of Isoagglutination is, in the present state of our knowledge, nil.

12. The only practical consideration associated with our present knowledge of this phenomenon is in relation to the determination of opsonic indices and amounts to this - that agglutinable red corpuscles are to be avoided in the making of opsonic mixtures.

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